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Somatic control of germline sexual development is mediated by the JAK/STAT pathway

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Abstract

Germ cells must develop along distinct male or female paths to produce the sperm or eggs required for sexual reproduction. In both mouse and *Drosophila*, sexual identity of germ cells is influenced by the sex of the surrounding somatic tissue (e.g. 1, 2; reviewed in 3, 4), but little is known about how the soma controls germline sex determination. Here we show that the JAK/STAT pathway provides a sex-specific signal from the soma to the germline in the *Drosophila* embryonic gonad. The somatic gonad expresses a JAK/STAT ligand, *unpaired* (*upd*), in a male-specific manner, and activates the JAK/STAT pathway in male germ cells at the time of gonad formation. Furthermore, the JAK/STAT pathway is necessary for male-specific germ cell behavior during early gonad development, and is sufficient to activate aspects of male germ cell behavior in female germ cells. This work provides direct evidence that the JAK/STAT pathway mediates a key signal from the somatic gonad that regulates male germline sexual development.

While investigating communication between the somatic gonad and germline, we found that the JAK/STAT pathway is specifically activated in male, but not female, germ cells. In *Drosophila*, JAK/STAT signaling (reviewed in 5) is initiated when an UPD or UPD-like ligand binds a transmembrane receptor (Domeless), activating the janus kinase (JAK), Hopscotch (HOP), which phosphorylates the STAT92E transcription factor. STAT activation has been shown to regulate *stat* gene expression 6 and can induce upregulation of the STAT92E protein, which can be used as an assay for JAK/STAT pathway activation. We found that STAT92E is upregulated specifically in male, but not female germ cells at the time of gonad formation (Fig. 1 a,b). This reflects male-specific activation of the JAK/STAT pathway since (i) the activated form of STAT92E (phospho-STAT92E) was also only detected in male germ cells (Fig. 1 c,d), and (ii) JAK activity is necessary and sufficient for STAT92E expression. Expression of a JAK inhibitor, *Socs36E* 7, resulted in loss of STAT92E expression in male germ cells (Fig. 1e) and expression of constitutively active JAK 8 (*hop^{TumL}*) induced STAT92E in female germ cells (Fig. 1f). The male-specific activation of STAT92E at this time is distinct from STAT92E activation in germ cells in the early embryo, which is not sex-specific and is regulated by the MAP kinase pathway 9.

We also found that STAT92E expression in male germ cells is dependent on their association with the somatic gonad. STAT92E was not detected in germ cells that were migrating to the gonad (Fig. 2a), but was detected in male germ cells after they contact the somatic gonad (Fig. 2b). STAT92E expression was greatly reduced or absent in *eya* mutants (data not shown), where somatic gonad identity is initiated, but not maintained 10. Furthermore, STAT92E was

not detected in germ cells found outside the gonad in wild type embryos (Fig. 2b, arrows) or in mis-localized germ cells in *wunen* (Fig. 2c) and *HMG-CoA reductase* (data not shown) mutants which lack guidance cues that target germ cells to the somatic gonad^{11, 12}. However, in these same mutants, STAT92E was detected in the few germ cells that contact the somatic gonad in male embryos (Fig. 2c inset).

STAT92E expression in the germline is dependent on the sex of the surrounding soma. When XX (normally female) germ cells were present in a soma that was masculinized by expression of the male form of the somatic sex determination gene *doublesex* (*dsx*)¹³, germ cells now expressed STAT92E (Fig. 2d). *dsx* does not play an autonomous role in germ cells themselves¹⁴, indicating that STAT92E induction in these embryos is caused by masculinization of the soma. Conversely, when the somatic gonad of an XY (normally male) embryo was feminized by expression of the sex determination gene *transformer* (*tra*) in the mesoderm, but not germ cells, STAT92E expression was no longer observed in XY germ cells (data not shown). Taken together, these data indicate that the male somatic gonad is necessary and sufficient to activate the JAK/STAT pathway in either XX or XY germ cells.

Consistent with this, we found that the JAK/STAT ligand, *upd*, is expressed specifically in the male, but not female, somatic gonad (Fig. 2 e,f). Expression of STAT92E in male germ cells was no longer detected in embryos in which *upd* and two homologs, *upd2* and *upd3*¹⁵, were deleted (*Df(os^{1a})*; Fig. 2g). Since male germ cells from embryos mutant for *upd* alone still expressed STAT92E (data not shown), JAK/STAT activation in the germline may be regulated redundantly by *upd* and one or more of its homologs. In addition, expression of *upd* in either the mesoderm (data not shown) or germ cells (Fig. 2h) was sufficient to induce STAT92E expression in XX germ cells. Expression of *upd2* or *upd3* was also capable of inducing STAT92E in germ cells (data not shown).

upd is also important for embryonic patterning¹⁶ and somatic sex determination^{17,18}. Interestingly, *upd* promotes female identity in the soma^{17,18}, but promotes male development in the germline (below). To verify that the effects of *upd* on the germline are not indirectly caused by other effects of *upd*, we examined indicators of embryonic segmentation (*Engrailed*), somatic sex determination (*Sex lethal*), somatic gonad identity (*Eyes absent*), and somatic gonad sexual identity (*Sox100B*) (data not shown). *Df(os^{1a})* hemizygous male embryos exhibited segmentation defects as expected¹⁶, but formed gonads that expressed normal somatic and sex-specific markers. Embryos ectopically expressing *upd* were normal in all respects examined.

We next addressed whether activation of the JAK/STAT pathway by the male somatic gonad regulates male-specific development of germ cells. In adult testes, the JAK/STAT pathway is required for maintenance of germline stem cells^{19,20}, making it difficult to assess the role of this pathway on male germ cell identity at this stage. Instead, we examined germ cells during embryogenesis and early larval stages, when germ cell development first becomes sexually dimorphic. In the mouse, the earliest manifestation of sex determination in the germline is differential regulation of the germline cell cycle by the soma⁴. In *Drosophila*, germ cells undergo 1–2 divisions after their formation, but are arrested in the cell cycle during germ cell migration and only resume division shortly after the gonad has formed^{21,22}. Since larval testes contain more germ cells than larval ovaries²³, we investigated if proliferation is regulated differently in male and female germ cells. Indeed, sex-specific analysis of a mitotic marker (phosphohistone-H3) in the germline indicated that germ cell proliferation is entirely male-specific during early stages of gonad development (Table 1). Furthermore, male-specific germ cell division is dependent on the male somatic gonad. Male germ cells did not proliferate in *eya* mutants that lack the somatic gonad, or in lost germ cells within *wunen* mutant embryos. XX germ cells in a masculinized soma (*dsx^D / dsx^L*) proliferated, while XY germ cells in a

feminized soma (*UAS-tra^F; twist-Gal4*) did not. Thus, the pattern of germ cell proliferation correlates exactly with activity of the JAK/STAT pathway in germ cells.

To assess whether JAK/STAT signaling regulates male-specific germ cell division, we examined embryos lacking zygotic *Stat92E* activity and observed a dramatic decrease in male germ cell proliferation (Table 1). Similar reductions in germ cell proliferation were observed in the *upd/upd-like* mutant (*Df(os^{1a})*) and in embryos where the JAK inhibitor *Socs36E* was expressed in germ cells. Thus, JAK/STAT activity is required within germ cells for proper male-specific germ cell division in the gonad. We also found that expression of *upd* in the germline was sufficient to induce proliferation in female germ cells. Thus, the JAK/STAT pathway can induce XX germ cells to exhibit this male-specific germ cell behavior.

We next investigated whether the JAK/STAT pathway regulates other aspects of male germ cell development. *male germline marker-1 (mgm-1)* is a *lacZ* enhancer trap line that is expressed in male germ cells, but not female germ cells, and therefore is a marker for male germ cell identity²⁴ (see Fig. 3 a,b,e,f). Inhibiting the JAK/STAT pathway by removing zygotic *Stat92E* activity did not affect *mgm-1* expression in the embryo (data not shown), which is as expected since initial *mgm-1* expression is dependent on germ cell autonomous cues²⁵. However, removal of zygotic *Stat92E* activity completely abolished *mgm-1* expression in first instar larvae. In wild-type first instar male larvae (Fig. 3a), *mgm-1* expression was observed in most germ cells, which are likely to be developing male germline stem cells and spermatogonia. No *mgm-1* expression was observed in *Stat92E*-mutant larvae (Fig 3c), and β -galactosidase expression was only observed in the soma, not the germline, in the pattern expected from the *Stat92E* P element allele (Fig 3d). In an experiment where 25% of larvae were expected to be both male and contain the *mgm-1* enhancer trap (see methods), 23.2% (n=262) of wild type larvae exhibited *mgm-1* expression in the germ cells, while no *Stat92E* mutant larvae (n=55) exhibited germ cell *mgm-1* expression (Fig 3c; this is significantly different than wild type siblings, $P=2.9 \times 10^{-5}$). Thus, *Stat92E* mutants exhibit a strong effect on male germline development, and some male germline cell types are either missing, or have an altered identity.

Finally, we assessed the extent to which activation of the JAK/STAT pathway can masculinize female germ cells. Female germ cells expressing *upd* are not expected to be fully masculinized since, although we are activating a male-specific signal, these germ cells are otherwise still in a female somatic environment and retain female germ cell autonomous cues. Indeed, such embryos gave rise to fertile adult females, indicating that at least some germ cells retain, or revert back to, a female identity (data not shown). This may be due, in part, to the failure of the *upd* construct to be expressed in the adult female germline²⁶. However, *upd* is sufficient to induce male-specific gene expression in embryonic XX germ cells. While *mgm-1* is normally expressed only in germ cells in males (Fig 3 e,f), *mgm-1* was expressed in all embryos (100%, n=71, Fig. 3g) when *upd* was ectopically expressed. In addition, we have identified two new male germline markers, *disc proliferation abnormal (dpa)* and *minichromosome maintenance 5 (mcm5)* (Fig 3 h,i and data not shown), that can also be induced by *upd*. Whereas these genes were normally expressed in germ cells only in males (*dpa*: 96% of males, n=56, 0% females, n=63; *mcm5*: 89.7% males, n=68, 0% females, n=70), female embryos exhibited germ cell expression of these genes when *upd* was ectopically expressed (Fig 3j and data not shown). In an experiment where only 50% of embryos are expected to express ectopic *upd* in the germline (see methods), 32.5% of female embryos expressed *dpa* (n=135) and 21.3% expressed *mcm5* (n=47). Therefore, *upd* expression is sufficient to activate male-specific gene expression in female germ cells.

Our data indicate that the JAK/STAT pathway mediates a critical signal from the male somatic gonad that is required for male germ cell development. This signal likely acts together with

male germ cell autonomous cues to promote male germline identity and spermatogenesis. This signal is also sufficient to activate the male pattern of proliferation and gene expression in female germ cells, even when these germ cells retain female germ cell autonomous cues and are present in an otherwise female soma. It will be very interesting in the future to identify additional (e.g. female) somatic signals, along with germ cell autonomous cues, and to assess the relative contribution of these factors to proper germline sexual development. Since one of the earliest aspects of sex-specific germ cell behavior in both *Drosophila* and mouse is the regulation of the germline cell cycle by the somatic gonad, it will be of further interest to determine if the somatic signals operating in *Drosophila* play a similar role in germline sex determination in mammals.

Methods

Fly stocks

w¹¹¹⁸, Canton-S and *ru st faf-lacZ e ca* flies were used as wild type. *twist-Gal4* and *24B-Gal4* (mesodermal drivers) and *nanos-Gal4::VP16* (germ cell driver) were used to express the following UAS constructs as indicated in the text: UAS-*upd* (M. Zeidler), UAS-*CG5988* (*upd2*), UAS-*CG5963* (*upd3*) and UAS-*hop^{TumL}* (D. Harrison), UAS-*Socs36E* (B. Mathey-Prevot), UAS-*tra^F* and UAS-*mCD8::GFP*. Other stocks include: *mgm-1* (M. Steinmann-Zwicky), *Stat92E⁰⁶³⁴⁶*, *Df(os^{1a})* (D. Harrison), *upd^{YM55}*, *wunen^{CE}* (K. Howard), *hmgcr^{clb1}*, *eya^{cli-III}*, *dsx^D* and *dsx^L*. Any unspecified stocks are from the Van Doren Lab or the Bloomington Stock Center (<http://flybase.bio.indiana.edu/>).

Immunocytochemistry, in situ hybridization & X-gal staining

Immunocytochemistry was performed as described for embryos²⁷ and larvae²⁸ except 0.1% Tween-20 was substituted for Triton X-100 during sonication and immunostaining of larvae. For quantitation of germ cell division, embryos were obtained for fixation from collections layed 6 hr at 25°C then aged 21 hr at 18°C. Larval analyses were conducted using 30-48 hr old larvae aged at 25°C. RNA *in situ* hybridization was performed as described²⁷ with hybridization carried out for 20–48 hrs and revealed using either colorimetric (NBT/BCIP) or fluorescent (HNPP, Roche) alkaline phosphatase substrates. *upd* anti-sense riboprobe was prepared by digesting **pBS-KS+*upd*** with HindIII, and transcribing with T7 polymerase (Promega) using digoxigenin labeled UTP (Boehringer-Mannheim). *dpa* and *mcm5* anti-sense riboprobes were generated by digesting **pFLC-1-*dpa*** or **pFLC-1-*mcm5*** (clones RE04051 and RE67590 from BACPAC Resources at CHORI) with SacI or SmaI respectively, then transcribing with T3 polymerase (Promega). X-gal staining of embryos was performed as described²⁹ with reactions proceeding for up to 48 hrs.

Antibodies

The following primary antibodies were used: chick anti-Vasa (K. Howard) at 1:10,000; rabbit anti-STAT92E (S. Hou) at 1:1,000; rabbit α-Phospho-STAT92E (Cell Signaling Technologies) at 1:50; rabbit anti-Phospho-Histone H3 (Upstate Biotechnology) at 1:1,000; rabbit α-Sox100B (S. Russell) at 1:1,000; rabbit anti-GFP (Torrey Pines) at 1:3,000; rabbit anti-β-gal (Cappel) at 1:10,000; mouse anti-β-gal (Promega) at 1:1,000; mouse anti-SXL-M18 (Developmental Studies Hybridoma Bank (DSHB; P. Schedl) at 1:300; mouse anti-EN-4D9 (DSHB; C. Goodman) at 1:2; mouse anti-EYA-10H6 (DSHB; N. Bonini) at 1:25; AP conjugated sheep anti-Digoxigenin (Roche) at 1:2,000. Secondary antibodies used at 1:500 include: Alexa488- and Alexa546-goat anti-rabbit, Alexa488- and Alexa568-goat anti-mouse; Alexa546-goat anti-chick (all from Molecular Probes), Cy5-goat anti-rabbit (Amersham-Pharmacia), and Cy5-goat anti-chick (Rockland).

Analysis of whole-mount embryos & larvae

Embryos and larvae were mounted in 70% glycerol in PBS with 0.1% Triton X-100 (Sigma) or 2.5% DABCO (Sigma) in 70% glycerol. Samples were viewed with a Zeiss Axioplan light microscope or a Zeiss 510 Meta confocal microscope. Staging of embryos was conducted according to Campos-Ortega and Hartenstein³⁰. Genotype of embryos was determined using GFP-expressing balancer chromosomes as described²⁷. Statistical analyses were conducted using a Student's t-Test assuming equal variance to compare test vs. control samples.

The sex of embryos and larvae was determined either by immunostaining with female-specific anti-SXL antibodies (Fig. 1 panels e,f; Fig. 2 panels a–d, h) or male specific anti-Sox100B antibodies (Fig. 3a–d), or by using an X chromosome carrying either P{Dfd-lacZ-HZ2.7} (W. McGinnis) (Fig. 1a–d; Fig. 3h–j) or FM7c, P{GAL4-Kr.C}, P{UAS-GFP.S65T} (Fig. 2e,f; Fig. 3e–g) (channels not shown in figures). Labelled X chromosomes were only present in male parents, and therefore only inherited by female embryos.

Embryos from mating #1 (below) were used to examine *mgm-1* expression in *Stat92E* mutant larvae. *Stat92E* mutants were identified by lack of GFP expression in the somatic gonad. These larvae were also labelled with anti-Sox100B to make sure that a normal fraction of male larvae were obtained. Embryos from mating #2 were used to determine the effects of *upd* expression on *mgm-1* expression in germ cells. *mgm-1/UAS-upd*; *nanos-Gal4/+* embryos were identified by *mgm-1* expression in the anterior soma, combined with lack of *ftz-lacZ* expression. Embryos from mating #3 were used to examine the effects of *upd* expression on *dpa* and *mcm-5* expression. Embryonic sex was determined by presence or absence of *Dfd-lacZ* expression.

Mating # 1:	♂	$\frac{mgm-1, CyO}{+}; \frac{Stat92E^{06346}}{TM3 Sb, Kr-GFP}$	$X^{\varnothing v}$	$\frac{Stat92E^{06346}}{TM3 Sb, Kr-GFP}$
Mating # 2:	♂	$\frac{mgm-1, CyO}{Sp}; \frac{nanos-Gal4}{nanos-Gal4}$	$X^{\varnothing v}$	$\frac{UAS-upd}{CyO, ftz-lacZ}$
Mating # 3:	♂	$\frac{Dfd-lacZ}{\rightarrow}; \frac{UAS-upd}{+}$	$X^{\varnothing v}$	$\frac{nanos-Gal4}{nanos-Gal4}$

Note: *Kr-GFP* = P{Gal4 - *Kr.C*}, P{UAS - GFP, S65T}

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References

- Steinmann-Zwicky M, Schmid H, Nothiger R. Cell-autonomous and inductive signals can determine the sex of the germ line of drosophila by regulating the gene Sxl. *Cell* 1989;57:157–66. [PubMed: 2702687]
- McLaren A. The fate of germ cells in the testis of fetal Sex-reversed mice. *J Reprod Fertil* 1981;61:461–7. [PubMed: 7205792]
- Oliver B. Genetic control of germline sexual dimorphism in *Drosophila*. *Int Rev Cytol* 2002;219:1–60. [PubMed: 12211627]
- McLaren A. Primordial germ cells in the mouse. *Dev Biol* 2003;262:1–15. [PubMed: 14512014]
- Hou SX, Zheng Z, Chen X, Perrimon N. The Jak/STAT pathway in model organisms: emerging roles in cell movement. *Dev Cell* 2002;3:765–778. [PubMed: 12479803]

6. Ichiba M, Nakajima K, Yamanaka Y, Kiuchi N, Hirano T. Autoregulation of the Stat3 gene through cooperation with a cAMP-responsive element-binding protein. *J Biol Chem* 1998;273:6132–8. [PubMed: 9497331]
7. Callus BA, Mathey-Prevot B. SOCS36E, a novel *Drosophila* SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc. *Oncogene* 2002;21:4812–4821. [PubMed: 12101419]
8. Harrison DA, Binari R, Nahreini TS, Gilman M, Perrimon N. Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *EMBO J* 1995;14:2857–2865. [PubMed: 7796812]
9. Li J, Xia F, Li WX. Coactivation of STAT and Ras is required for germ cell proliferation and invasive migration in *Drosophila*. *Dev Cell* 2003;5:787–98. [PubMed: 14602078]
10. Boyle M, Bonini N, DiNardo S. Expression and function of clift in the development of somatic gonadal precursors within the *Drosophila* mesoderm. *Development* 1997;124:971–82. [PubMed: 9056773]
11. Zhang N, Zhang J, Purcell KJ, Cheng Y, Howard K. The *Drosophila* protein Wunen repels migrating germ cells. *Nature* 1997;385:64–67. [PubMed: 8985246]
12. Van Doren M, Broihier HT, Moore LA, Lehmann R. HMG-CoA reductase guides migrating promordial germ cells. *Nature* 1998;396:466–469. [PubMed: 9853754]
13. Nagoshi RN, Baker BS. Regulation of sex-specific RNA splicing at the *Drosophila* doublesex gene: cis-acting mutations in exon sequences alter sex-specific RNA splicing patterns. *Genes Dev* 1990;4:89–97. [PubMed: 2155161]
14. Schupbach T. Autosomal mutations that interfere with sex determination in somatic cells of *Drosophila* have no direct effect on the germline. *Dev Biol* 1982;89:117–27. [PubMed: 7054003]
15. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev Cell* 2003;5:441–50. [PubMed: 12967563]
16. Harrison DA, McCoon PE, Binari R, Gilman M, Perrimon N. *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes Dev* 1998;12:3252–63. [PubMed: 9784499]
17. Jinks TM, Polydorides AD, Calhoun G, Schedl P. The JAK/STAT signaling pathway is required for the initial choice of sexual identity in *Drosophila melanogaster*. *Molecular Cell* 2000;5:581–587. [PubMed: 10882142]
18. Sefton L, Timmer JR, Zhang Y, Beranger F, Cline TW. An extracellular activator of the *Drosophila* JAK/STAT pathway is a sex-determination signal element. *Nature* 2000;405:970–973. [PubMed: 10879541]
19. Tulina N, Matunis E. Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. *Science* 2001;294:2546–2549. [PubMed: 11752575]
20. Kiger AA, Jones DL, Schultz C, Rogers MB, Fuller MT. Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* 2001;294:2542–2545. [PubMed: 11752574]
21. Sonnenblick BP. Germ cell movements and sex differentiation of the gonads in the *Drosophila* embryo. *Proc Natl Acad Sci USA* 1941;26:373–81. [PubMed: 16588366]
22. Asaoka-Taguchi M, Yamada M, Nakamura A, Hanyu K, Kobayashi S. Maternal Pumilio acts together with Nanos in germline development in *Drosophila* embryos. *Nature Cell Biology* 1999;1:431–437.
23. Kerkis J. The growth of the gonads in *Drosophila melanogaster*. *Genetics* 1931;16:212–244. [PubMed: 17246617]
24. Staab S, Heller A, Steinmann-Zwicky M. Somatic sex-determining signals act on XX germ cells in *Drosophila* embryos. *Development* 1996;122:4065–4071. [PubMed: 9012526]
25. Janzer B, Steinmann-Zwicky M. Cell-autonomous and somatic signals control sex-specific gene expression in XY germ cells of *Drosophila*. *Mech Dev* 2001;100:3–13. [PubMed: 11118879]
26. Rorth P. Gal4 in the *Drosophila* female germline. *Mech Dev* 1998;78:113–8. [PubMed: 9858703]
27. DeFalco TJ, et al. Sex-specific apoptosis regulates sexual dimorphism in the *Drosophila* embryonic gonad. *Dev Cell* 2003;5:205–216. [PubMed: 12919673]
28. Patel, N. in *Drosophila melanogaster: Practical Uses in Cell and Molecular Biology* (eds. Goldstein, L. S. B. & Fyrberg, E. A.) 445–487 (Academic Press, San Diego, 1994).

29. Moore LA, Broihier HT, Van Doren M, Lunsford LB, Lehmann R. Identification of genes controlling germ cell migration and embryonic gonad formation in *Drosophila*. *Development* 1998;125:667–78. [PubMed: 9435287]
30. Campos-Ortega, J. & Hartenstein, V. *The embryonic development of Drosophila melanogaster* (Springer-Verlag, New York, 1985).

Figure 1.

The Jak/Stat pathway is activated in male germ cells. **a-f.** Embryonic gonads (stage 15) co-immunolabeled with anti-Vasa (red) and either anti-STAT92E (**a, b, e, f**, green) or anti-phospho-STAT92E (**c, d**, green). Smaller panels show red (') or green (") channels separately. Sex was determined in this and subsequent figures as described (methods). **a-d.** Wild-type male (**a,c**) and female (**b,d**) gonads. **e.** Male embryo with germ cells expressing *Socs36E* (UAS-*Socs36E*; *nanos-Gal4*). **f.** Female embryos with germ cells expressing *hop^{TumL}* (UAS-*hop^{TumL}*; *nanos-Gal4*). Scale bar (12.5µm) in panel **a** applies to panels **a-f**.

Figure 2.

The Jak/Stat pathway is activated by the male somatic gonad. Embryos are Stage 15 and wild type unless noted. **a–d**, anti-Vasa (red), anti-STAT92E (green). **a,b**, Males stage 11 (**a**) and stage 13 (**b**). Gonad (outline) and mis-localized germ cells (arrows) are indicated. **c**, Mis-localized germ cells in male *wunen^{CE}* mutant (inset: germ cells in the gonad of the same embryo). **d**, XX germ cells in a male soma (*dsx^{D/dsx^L}*). **e,f**, *upd* riboprobe (red) and anti-Vasa (green). *upd* is expressed in (**e**) male and not (**f**) female gonads. **g,h**, anti-Vasa (red), anti-STAT92E (green). Gonads in (**g**) XY Df(*os^{1a}*) embryo and (**h**) female embryo with germ cells expressing *Upd* (*UAS-upd; nanos-Gal4*). Scale bars (12.5μm) in **a** and **d** apply to **a–c** or **d–h**, respectively.

Figure 3.

JAK/STAT activation regulates male-specific germ cell behavior. **a–d.** Larval (L1) gonads. Anti-Vasa (red), anti-β-Gal (green). **a,b.** *mgm-1* expression in (a) male and (b) female from *mgm-1/+* X *Kr-GFP/Y*. **c.** Male *Stat92E⁰⁶³⁴⁶* mutant from mating #1 (methods) with no *mgm-1* expression in the germ cells. **d.** Male *Stat92E⁰⁶³⁴⁶ /+* showing somatic *Stat92E⁰⁶³⁴⁶* enhancer trap expression. **e–g.** *mgm-1* expression (X-gal staining) in stage 16 gonads (circled) in (e) male, (f) female and (g) embryo expressing ectopic *upd* (mating #2, methods). **h–j.** *dpa* expression (*in situ* hybridization) in stage 17 gonads (circled) in (h) male, (i) female and (j) female embryo expressing ectopic *upd* (mating #3, methods). Scale bars (12.5μm) in a and e apply to a–d and e–j, respectively.

Table 1

Germ cell division in embryonic gonads is male-specific and regulated by the JAK/STAT pathway.

Genotype	% XY embryos with 1 or more dividing germ cells (n)	% XX embryos with 1 or more dividing germ cells (n)
wild type ¹	28.0 (147)	0 (170)
<i>eyes absent</i>	0 (69)	ND
<i>wunen</i> - mis-localized germ cells ²	0 (42)	ND
<i>wunen</i> - gonadal germ cells ²	33.0 (42)	ND
<i>dsx^D/dsx^L</i> - masculinized soma	39.0 (41)	24 (33)
<i>twist-Gal4</i> / <i>UAS-tra^F</i> - feminized soma	0 (42)	0 (40)
<i>Stat92E⁰⁶³⁴⁶</i>	4.0 (101)	0 (90)
control ³	28.0 (232)	0 (214)
<i>UAS-Socs36E; nanos-Gal4</i>	6.3 (111)	0 (103)
control ⁴	38.8 (85)	0 (88)
<i>Df(os^{1a})</i>	7.5 (94)	NA ⁴
control ³	35.1 (94)	ND
<i>UAS-upd; nanos-Gal4</i>	55.0 (40)	19.0 (58)
control ³	21.7 (46)	0 (46)

¹ w1118 (n=108) and *faf-lacZ* (n=209)

² Mis-localized germ cells and germ cells in gonads examined separately

³ Wild-type siblings

⁴ *UAS-mCD::GFP* driven by the *nanos-Gal4* driver

^ψ P-value according to Student's t-Test (see methods)

⁴ Not applicable; deficiency on X chromosome